(substrate and sodium ions in the core) were performed, in a fully atomistic, solvated bilayer environment. In the Apo_MD and the Na_MD simulations, the HP1 loop has a much higher mobility than the HP2 loop. However, the release of the substrate into the intracellular solvent in Asp_Na_MD, required the motions of both the HP1 and the HP2 loops. The opening up of HP2 loop facilitates solvation of the binding site resulting in the substrate being dislodged from its position. Prior to substrate release, the HP1 loop moves further down into the solvent, exposing the HP1-tip and the substrate to the solvent, followed by its subsequent release into the intracellular solvent. These results suggest that the intracellular gating involves sequential opening of both HP1 and HP2 loops.

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Ligand Exit and Entry Pathways for Monoamine Transporters

Bonnie A. Merchant, Jeffry D. Madura.

The monoamine transporters are targets for various medicinal and illegal drugs that affect mood and behavior. Of particular interest are the dopamine (DAT) and serotonin (SERT) transporters of which the three-dimensional structures are unknown. A three-dimensional structure homologous to DAT and SERT, both in sequence and in function, is the leucine transporter ($LeuT_{Aa}$). While there is significant binding and uptake data, some structural information and homology models, there is no clear understanding of the transport pathways for ligands of $LeuT_{Aa}$, DAT or SERT. The Random Acceleration Molecular Dynamics (RAMD) method as implemented in NAMD, was used to study the entry and exit pathways of various chemically relevant substrates in $LeuT_{Aa}$ and a homology model of DAT. Example pathways as illustrated in Figure 1. Free energy scores of the pathways have been characterized via the Multi-Configuration Thermodynamic Integration method. Several sites of low free energy score have been indentified, which correspond to primary and secondary substrate pockets of the transporters. Detailed free energy and structural results of the transport pathways will be presented.

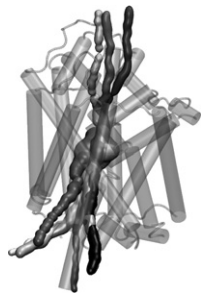

Figure 1. Representative transport pathways of leucine through LeuTAa using RAMD.

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Proton Transport and Conformational Changes in H+/CL- Exchangers

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CLC transporters localize to the membranes of intracellular compartments, such as lysosomes and endosomes, where they mediate a variety of physiological roles by exchanging 2 Cl^- ions for 1 H^+. Very little is known about the mechanisms underlying the transport process and many questions are still unanswered, in particular the H^+ pathway has not been identified and which conformational changes the transporters undergo is still not clear. To elucidate these important aspects we decided to investigate how protons translocate through the protein by studying the deuterium kinetic isotope effect and to probe the conformational changes by measuring the temperature dependence of the transport rate of the human transporter CLC-5 and the bacterial homologue CLC-ec1. We found that both CLC-5 and CLC-ec1 have similar thermodynamic profile. The transport rate in deuterium is decreased by ~20-40%, suggesting a H^+ movement through a hydrogen-bonded pathway, possibly formed by water. Both transporters also have a similar and modest temperature dependence, suggesting that the proteins undergo limited conformational changes. Interestingly, we observed unaltered apparent activation enthalpy of transport when Cl^- or H^+ binding, coupling or transporter gating are impaired, while the transport rates are affected. We hypothesize that H^+ movement through a CLC transporter takes place along a series of hydrogen bond formed by water molecules and that only limited conformational changes occur during the transport cycle. Finally, we propose a transport mechanism where several rate-limiting steps with similar apparent activation enthalpies are involved, instead of a single rate-limiting step mechanism.

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Analysis of the Oligomeric State of Surface-Localized Proton-Coupled Folate Transporter by Blue Native Polyacrylamide Gel Electrophoresis

Prachi Nakashe, Michaela Jansen.

Folate vitamins are essential for DNA replication and cellular proliferation. However, mammalian organisms are devoid of de novo folate biosynthesis and thus rely on dietary sources to meet their metabolic needs. The proton coupled folate transporter (PCFT/SLC46A1) has been recently identified as the molecular entity of the carrier mediated intestinal folate uptake pathway for folic acids from food sources. PCFT is also involved in the absorption of chemotherapeutically used antifolates. Currently, there is limited information about the structure and function of PCFT. Hydropathy analysis suggests that there are 10-12 transmembrane segments. Further, using the Substituted Cysteine Accessibility Method (SCAM) evidence was provided for a 12 transmembrane segment topology. Blue Native Polyacrylamide Gel Electrophoresis (BN-PAGE) is a technique for separation of protein complexes in a native state with high resolution. We expressed PCFT in Xenopus laevis oocytes. Oocyte plasma membranes were polymerized to the vitelline membrane using ludox colloidal silica solution and polyacrylic acid, isolated by centrifugation, and plasma membrane proteins subsequently solubilized with digitonin and separated by BN-PAGE. The separation characteristics of native PCFT were compared to a molecular ruler produced by partial dissociation of homopentameric 5-hydroxytryptamine type 3A (5HT3A) receptors. Under native conditions, 5HT3A subunits largely migrated as a pentamer and PCFT only as a monomer. Treatment with denaturing agents generated a ladder of five bands for 5HT3A subunits, which consisted of monomer, dimer, trimer, tetramer and pentamer. Addition of crosslinking agents resulted in migration of 5HT3A subunits as a pentamer, even in the presence of denaturing agents. In contrast, crosslinking agents did not induce oligomeric assemblies of PCFT. These results indicate that functional plasma-membrane bound PCFT is a monomeric protein.

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Sets of Local Entropy-Enthalpy Change Leads to Global Entropy-Enthalpy Change in SERCA

Anu Nagarajan, Thomas B. Woolf.

Major conformational changes are involved in the multi-step catalytic cycle of the sarcoplasmic endoplasmic reticulum calcium ATPase (SERCA) pump. The movement and rotation of the Actuator (A) domain is crucial for ion translocation. The A-domain is connected to the trans-membrane helices through linker regions. Mutational studies on the A-M3 linker region show that varying the length of this region causes significant changes in the rate of the conformational transitions (JBC (2009),284,12258-12265). The focus of this research is to study the impact of these mutations on the structural changes during the transition from E1 to E2, the behavior of the A-M3 linker region, and the overall rate of the conformational transitions. In order to achieve faster computation of transitions, we implemented the MARTINI coarse-grained protein and lipid model in CHARMM. We used Dynamic Importance Sampling (DIMS) to compute transitions from the E1 to the E2 state for both directions of each mutant in both coarse-grained and all atom models. Analysis of the transitions across mutants shows that the angle formed by A-P-N domains changes by up to 20 degrees with an increasing number of inserts into the A-M3 linker region. Estimates of barrier crossing time from the simulation and experimental values are highly correlated (R^2=0.934). Quasiharmonic analysis on the domains, linkers and transmembrane helices show entropic changes between the mutants and compensation effects. Interaction energies of the same regions indicate entropic-enthalpic compensation. Further investigation of the end state simulations shows changes in the number of high density water sites around the A-M3 linker region across the mutants. The varying degree of the change in volume due to water sites across all the mutants indicates a ripple effect where the local entropy-enthaply changes translate to global entropy-enthalpy changes.

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Functional Analysis of Transmembrane Domain 3 in NKCC1

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We sought to determine the functional roles of residues in transmembrane domain (TM) 3 of human NKCC1 using tryptophan and cysteine scanning mutagenesis. We generated a structural alignment of the transmembrane domains of NKCC1 to the related APC transporters ApcT and AdiC, and obtained 3-D alignments using Modeller. Based on these alignments, residues 368 to 380 were predicted to form part of the inner 2/3 of the translocation pore. We substituted these residues with tryptophan and cysteine and determined the impact of the changes on protein synthesis and cell surface delivery by Western blotting and immunofluorescence microscopy. Most of our mutants expressed and localized similar to wild type NKCC1 and these were analyzed in depth for transporter function by means of Rb^{86} influx assays. A working hypothesis is that tryptophan mutants that are much reduced in function are too hydrophobic for the solvent interface and those residues that retain function are either protein interior or lipid facing. The pattern of the tryptophan scan followed an alpha helical periodicity. Based on the tryptophan scan we deduced that the non-functional mutants I368W, G369W, F372W, A375W, N376W, A379W are pore lining residues. Cysteine scanning complemented the results of the tryptophan scan. Since cysteine is a mild mutation, most mutants were functional. However I368C, G369C, A379C showed dramatic reduction or loss of function